

Application No.: 10/734,600  
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Reply to Office Action of January 4, 2005

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

Please withdraw claims 1-48.

Please amend Claims 49, 61, 63, and 67-72.

Please add new claims 73-110.

A complete listing of the claims is listed below with proper claim identifiers.

1. (Withdrawn) A solution, for cryopreservation of a tissue, comprising:
  - water;
  - a bio-compatible buffer;
  - a cell-impermeant constituent;
  - a cell-permeant constituent; and
  - a radical scavenger.
2. (Withdrawn) The solution of claim 1, wherein said tissue is selected from the group consisting of partial organs, blood cells, blood proteins, heart valve leaflets, aortic roots, aortic walls, pulmonary valves, pulmonary conduits, non-valved conduits, mitral valves, monocusps, tendons, ligaments, fascia, blood vessels, arteries, veins, ureters, diaphragm, pericardium, umbilical cords, dura matter membranes, and tympanic membranes.
3. (Withdrawn) The solution of claim 1, wherein said tissue is selected from the group consisting of heart valves, pulmonary conduits, monocusps, blood vessels, and tendons.
4. (Withdrawn) The solution of claim 1, wherein said tissue is a tendon.
5. (Withdrawn) The solution of claim 1, wherein said tissue is a heart valve.

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6. (Withdrawn) The solution of claim 1, wherein said tissue is a pulmonary conduit.
7. (Withdrawn) The solution of claim 1, wherein said tissue is a non-valved conduit.
8. (Withdrawn) The solution of claim 1, wherein said tissue is a monocusp.
9. (Withdrawn) The solution of claim 1, wherein said tissue is decellularized.
10. (Withdrawn) The solution of claim 1, wherein said tissue comprises at least 50% water and at least 8% solids by weight.
11. (Withdrawn) The solution of claim 1, wherein said bio-compatible buffer maintains the pH of said solution from about 6 to about 8 before, during, and after freezing.
12. (Withdrawn) The solution of claim 1, wherein said bio-compatible buffer is isotonic.
13. (Withdrawn) The solution of claim 1, wherein said bio-compatible buffer comprises a buffer selected from the group consisting of phosphate-buffered saline, N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) buffered saline, morpholine propanesulfonic acid buffered saline, tris(hydroxymethyl) aminomethane buffered saline, borate buffered saline, bicarbonate buffered saline, carbonate buffered saline, cacodylate buffered saline, citrate ion buffered saline, and mixtures thereof.
14. (Withdrawn) The solution of claim 1, wherein said bio-compatible buffer comprises a buffer selected from the group consisting of phosphate-buffered saline, N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) buffered saline, tris(hydroxymethyl) aminomethane buffered saline, and mixtures thereof.

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15. (Withdrawn) The solution of claim 1, wherein said bio-compatible buffer comprises phosphate-buffered saline.
16. (Withdrawn) The solution of claim 15, wherein said bio-compatible buffer maintains the pH of said solution from about 7 to about 8.
17. (Withdrawn) The solution of claim 15, wherein said bio-compatible buffer maintains the pH of said solution at about 7.4.
18. (Withdrawn) The solution of claim 1, wherein said bio-compatible buffer has a concentration of from 15 to 100 mM in said solution.
19. (Withdrawn) The solution of claim 1, wherein said bio-compatible buffer has a concentration of from 20 to 75 mM in said solution.
20. (Withdrawn) The solution of claim 1, wherein said bio-compatible buffer further comprises sodium chloride.
21. (Withdrawn) The solution of claim 20, wherein said sodium chloride has a concentration of from 0.02 to 0.5 M in said solution.
22. (Withdrawn) The solution of claim 20, wherein said sodium chloride has a concentration of about 0.154 M in said solution.
23. (Withdrawn) The solution of claim 1, wherein said cell-impermeant constituent is selected from the group consisting of proteins, serums, monosaccharides, sucrose, polysaccharides, dextran, agrose, alginate, long-chain polymers, polyvinylpyrrolidone, hydroxyethyl starch, derivatives thereof, and mixtures thereof.

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24. (Withdrawn) The solution of claim 1, wherein said cell-impermeant constituent is selected from the group consisting of polyvinylpyrrolidones, hydroxyethyl starches, their derivatives, and mixtures thereof.
25. (Withdrawn) The solution of claim 1, wherein said cell-impermeant constituent comprises a polyvinylpyrrolidone.
26. (Withdrawn) The solution of claim 25, wherein said polyvinylpyrrolidone has a molecular weight of about 17,000.
27. (Withdrawn) The solution of claim 1, wherein said cell-impermeant constituent comprises from 5 to 30 % of said solution.
28. (Withdrawn) The solution of claim 1, wherein said cell-impermeant constituent comprises from 10 to 14 % of said solution.
29. (Withdrawn) The solution of claim 1, wherein said cell-permeant constituent is selected from the group consisting of alcohols, mannitol, propanediol, isopropanol, ethanol, t-butanol, glycerol, glycols, ethylene glycol, propylene glycol, trimethylamine acetate, aldoses, ketones, xylose, erythrose, arabinose, ribose, glucose, fructose, galactose, and mixtures thereof.
30. (Withdrawn) The solution of claim 1, wherein said cell-permeant constituent is selected from the group consisting of isopropanol, ethanol, and mixtures thereof.
31. (Withdrawn) The solution of claim 1, wherein said cell-permeant constituent comprises isopropanol.

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32. (Withdrawn) The solution of claim 1, wherein said cell-permeant constituent comprises from 5 to 30 % of said solution.
33. (Withdrawn) The solution of claim 1, wherein said cell-permeant constituent comprises about 15 % of said solution.
34. (Withdrawn) The solution of claim 1, wherein said radical scavenger is selected from the group consisting of sodium ascorbate, carotenoids, 1-ascorbic acid, d-isoascorbic acid, sodium sulfite, sodium metabisulfite, sulfur dioxide, nicotinic acid, nicotinic acid amine, cysteine, glutathione, sodium nitrate, sodium nitrite, flavenoids, selenium, alpha-lipoic acids, acetyl cysteine, water-soluble tocopherol derivatives, analogs thereof, isomers thereof, derivatives thereof, and mixtures thereof.
35. (Withdrawn) The solution of claim 1, wherein said radical scavenger is selected from the group consisting of sodium ascorbate, water-soluble derivatives of ascorbate, cysteine, Lazaroids, carotenoids, and mixtures thereof.
36. (Withdrawn) The solution of claim 1, wherein said radical scavenger comprises sodium ascorbate.
37. (Withdrawn) The solution of claim 1, wherein said radical scavenger combines with radicals generated by a gamma irradiation of said solution.
38. (Withdrawn) The solution of claim 1, wherein said radical scavenger has a concentration of from 0.1 to 1.5 M in said solution.
39. (Withdrawn) The solution of claim 1, wherein said radical scavenger has a concentration of about 0.5 M in said solution.

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40. (Withdrawn) An irradiated biological tissue product, for implantation in a mammal after storage, comprising:
- a package;
  - a tissue;
  - a bio-compatible buffer;
  - a cell-impermeant constituent;
  - a cell-permeant constituent; and
  - a radical scavenger.
41. (Withdrawn) The tissue of claim 40, wherein said tissue is irradiated with gamma radiation.
42. (Withdrawn) The tissue of claim 40, wherein said tissue is stored for at least three days prior to implantation.
43. (Withdrawn) The tissue of claim 40, wherein said tissue is stored for from one week to one year prior to implantation.
44. (Withdrawn) The tissue of claim 40, wherein said tissue is frozen.
45. (Withdrawn) The tissue of claim 40, wherein said tissue is at a cryogenic temperature.
46. (Withdrawn) The tissue of claim 40, wherein said tissue is at a temperature lower than -70° C.
47. (Withdrawn) The tissue of claim 40, wherein said tissue is at a temperature from about 0° C to about 15° C.

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48. (Withdrawn) The tissue of claim 40, wherein after thawing, said tissue has a denaturation temperature that is within about  $\pm 3^{\circ}$  C of a denaturation temperature of the same type of native tissue.

49. (Currently amended) A method, for forming a cryopreserved tissue, comprising:  
providing a solution comprising water, a bio-compatible buffer, a cell-impermeant constituent, a cell-permeant constituent, and a radical scavenger;  
combining the solution with a tissue; and  
freezing the solution and the tissue.

50. (Original) The method of claim 49, wherein the tissue and solution are placed in a package before freezing.

51. (Original) The method of claim 49, wherein said solution is degassed.

52. (Original) The method of claim 49, further comprising irradiating said cryopreserved tissue with ionizing radiation to form an irradiated cryopreserved tissue.

53. (Original) The method of claim 52, further comprising thawing said irradiated cryopreserved tissue after the irradiated cryopreserved tissue was subjected to extended storage.

54. (Original) The method of claim 52, wherein said ionizing radiation is an electron beam.

55. (Original) The method of claim 52, wherein said ionizing radiation is gamma radiation.

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56. (Original) The method of claim 55, wherein said gamma radiation is administered to said cryopreserved tissue at a rate from about 0.38 to 0.45 Mrad/hr.
57. (Original) The method of claim 55, wherein a total of from 5,000 to 8,000,000 rads is administered to said cryopreserved tissue.
58. (Original) The method of claim 55, further comprising thawing said sterilized cryopreserved tissue, wherein after thawing, the thawed tissue has a denaturation temperature within about  $\pm 3^{\circ}$  C of a denaturation temperature of the same type of native tissue.
59. (Original) The method of claim 49, further comprising packaging said cryopreserved tissue.
60. (Original) The method of claim 59, wherein after said packaging, said cryopreserved tissue is sterilized.
61. (Currently Amended) The method of claim 49, further comprising lowering the temperature of said solution and said tissue to at least  $-18^{\circ}$  C.
62. (Original) The method of claim 49, further comprising lowering the temperature of said solution and said tissue to at least the freezing point of the solution.
63. (Currently Amended) The method of claim 49, further comprising lowering the temperature of said solution and said tissue to at least  $-130^{\circ}$  C.
64. (Original) The method of claim 49, further comprising lowering the temperature of said solution and said tissue to at least the glass transition temperature of the solution.



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65. (Original) The method of claim 49, further comprising lowering the temperature of said solution and said tissue to a cryogenic temperature.

66. (Original) In a process for cryopreserving a tissue, wherein said tissue is combined with a cryoprotectant solution, frozen, and sterilized with ionizing radiation, the improvement comprising a cryoprotectant solution comprising:

from 15 to 100 mM of a bio-compatible buffer;

from 5 to 30 % of a cell-impermeant constituent comprising polyvinylpyrrolidone;

from 5 to 30 % of a cell-permeant constituent comprising isopropanol;

and

from 0.1 to 1.5 M of a radical scavenger comprising sodium ascorbate.

67. (Currently amended) The [process] improvement of claim 66, wherein said bio-compatible buffer is isotonic.

68. (Currently amended) The [process] improvement of claim 66, wherein said bio-compatible buffer comprises phosphate-buffered saline.

69. (Currently amended) The [process] improvement of claim 66, wherein said solution comprises from 20 to 75 mM of said bio-compatible buffer.

70. (Currently amended) The [process] improvement of claim 66, wherein said solution comprises from 10 to 14 % of said cell-impermeant constituent.

71. (Currently amended) The [process] improvement of claim 66, wherein said solution comprises about 15 % of said cell-permeant constituent.

72. (Currently amended) The [process] improvement of claim 66, wherein said solution comprises about 0.5 M of said radical scavenger.

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73. (New) The method of claim 49, wherein the tissue is selected from the group consisting of partial organs, blood cells, blood proteins, heart valve leaflets, aortic roots, aortic walls, pulmonary valves, pulmonary conduits, non-valved conduits, mitral valves, monocusps, tendons, ligaments, fascia, blood vessels, arteries, veins, ureters, diaphragm, pericardium, umbilical cords, dura matter membranes, and tympanic membranes.

74. (New) The method of claim 49, wherein the tissue is selected from the group consisting of heart valves, pulmonary conduits, monocusps, blood vessels, and tendons.

75. (New) The method of claim 49, wherein the tissue is a tendon.

76. (New) The method of claim 49, wherein the tissue is a heart valve.

77. (New) The method of claim 49, wherein the tissue is a pulmonary conduit.

78. (New) The method of claim 49, wherein the tissue is a non-valved conduit.

79. (New) The method of claim 49, wherein the tissue is a monocusp.

80. (New) The method of claim 49, wherein the tissue is decellularized.

81. (New) The method of claim 49, wherein the bio-compatible buffer maintains the pH of said solution from about 6 to about 8 before, during, and after freezing.

82. (New) The method of claim 49, wherein the bio-compatible buffer is isotonic.

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83. (New) The method of claim 49, wherein the bio-compatible buffer comprises a buffer selected from the group consisting of phosphate-buffered saline, N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) buffered saline, morpholine propanesulfonic acid buffered saline, tris(hydroxymethyl) aminomethane buffered saline, borate buffered saline, bicarbonate buffered saline, carbonate buffered saline, cacodylate buffered saline, citrate ion buffered saline, and mixtures thereof.

84. (New) The method of claim 49, wherein the bio-compatible buffer comprises a buffer selected from the group consisting of phosphate-buffered saline, N-(2-hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) buffered saline, tris(hydroxymethyl) aminomethane buffered saline, and mixtures thereof.

85. (New) The method of claim 49, wherein the bio-compatible buffer comprises phosphate-buffered saline.

86. (New) The method of claim 85, wherein the bio-compatible buffer maintains the pH of said solution from about 7 to about 8.

87. (New) The method of claim 85, wherein the bio-compatible buffer maintains the pH of said solution at about 7.4.

88. (New) The method of claim 49, wherein the bio-compatible buffer has a concentration of from 15 to 100 mM in said solution.

89. (New) The method of claim 49, wherein the bio-compatible buffer has a concentration of from 20 to 75 mM in said solution.

90. (New) The method of claim 49, wherein the bio-compatible buffer further comprises sodium chloride.

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91. (New) The method of claim 90, wherein the sodium chloride has a concentration of from 0.02 to 0.5 M in said solution.

92. (New) The method of claim 90, wherein the sodium chloride has a concentration of about 0.154 M in said solution.

93. (New) The method of claim 49, wherein the cell-impermeant constituent is selected from the group consisting of proteins, serums, monosaccharides, sucrose, polysaccharides, dextran, agrose, alginate, long-chain polymers, polyvinylpyrrolidone, hydroxyethyl starch, derivatives thereof, and mixtures thereof.

94. (New) The method of claim 49, wherein the cell-impermeant constituent is selected from the group consisting of polyvinylpyrrolidones, hydroxyethyl starches, their derivatives, and mixtures thereof.

95. (New) The method of claim 49, wherein the cell-impermeant constituent comprises a polyvinylpyrrolidone.

96. (New) The method of claim 49, wherein the cell-impermeant constituent comprises from 5 to 30 % of the solution.

97. (New) The method of claim 49, wherein the cell-impermeant constituent comprises from 10 to 14 % of the solution.

98. (New) The method of claim 49, wherein the cell-permeant constituent is selected from the group consisting of alcohols, mannitol, propanediol, isopropanol, ethanol, t-butanol, glycerol, glycols, ethylene glycol, propylene glycol, trimethylamine acetate, aldoses, ketones, xylose, erythrose, arabinose, ribose, glucose, fructose, galactose, and mixtures thereof.

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99. (New) The method of claim 49, wherein the cell-permeant constituent is selected from the group consisting of isopropanol, ethanol, and mixtures thereof.

100. (New) The method of claim 49, wherein the cell-permeant constituent comprises isopropanol.

101. (New) The method of claim 49, wherein the cell-permeant constituent comprises from 5 to 30 % of said solution.

102. (New) The method of claim 49, wherein the cell-permeant constituent comprises about 15 % of said solution.

103. (New) The method of claim 49, wherein the radical scavenger is selected from the group consisting of sodium ascorbate, carotenoids, 1-ascorbic acid, d-isoascorbic acid, sodium sulfite, sodium metabisulfite, sulfur dioxide, nicotinic acid, nicotinic acid amine, cysteine, glutathione, sodium nitrate, sodium nitrite, flavenoids, selenium, alpha-lipoic acids, acetyl cysteine, water-soluble tocopherol derivatives, analogs thereof, isomers thereof, derivatives thereof, and mixtures thereof.

104. (New) The method of claim 49, wherein said radical scavenger is selected from the group consisting of sodium ascorbate, water-soluble derivatives of ascorbate, cysteine, Lazaroids, carotenoids, and mixtures thereof.

105. (New) The method of claim 49, wherein said radical scavenger comprises sodium ascorbate.

106. (New) The method of claim 49, wherein said radical scavenger combines with radicals generated by a gamma irradiation of said solution.

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107. (New) The method of claim 49, wherein said radical scavenger has a concentration of from 0.1 to 1.5 M in said solution.

108. (New) The method of claim 49, wherein said radical scavenger has a concentration of about 0.5 M in said solution.

109. (New) The method of claim 49, wherein the solution comprises from 15 to 100 mM of a bio-compatible buffer; from 5 to 30 % of the cell-impermeant constituent comprising polyvinylpyrrolidone; from 5 to 30 % of the cell-permeant constituent comprising isopropanol; and from 0.1 to 1.5 M of a radical scavenger comprising sodium ascorbate.

110. (New) The method of claim 109, further comprising irradiating the cryopreserved tissue with ionizing radiation to form an irradiated cryopreserved tissue.